Current Biology, Vol. 13, 1740–1745, September 30, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.cub.2003.08.053

Cell Cycle-Regulated Transcription through the FHA Domain of Fkh2p and the Coactivator Ndd1p

Zoulfia Darieva,[1,5] Aline Pic-Taylor,[2,5] Joanna Boros,[1,3] Adonis Spanos,[4] Marco Geymonat,[4] Richard J. Reece,[1] Steven G. Sedgwick,[4] Andrew D. Sharrocks,[1,*] and Brian A. Morgan[2,*]

[1]School of Biological Sciences
University of Manchester
2.205 Stopford Building
Oxford Road
Manchester, M13 9PT

[2]School of Cell and Molecular Biosciences
Faculty of Medical Sciences
University of Newcastle upon Tyne
Newcastle upon Tyne, NE2 4HH
United Kingdom

[3]Department of Genetics
University of Warsaw
ul. Pawinskiego 5A
02-106 Warsaw
Poland

[4]Division of Yeast Genetics
NIMR
Mill Hill
London, NW7 1AA
United Kingdom

Summary

Recent studies in *Saccharomyces cerevisiae* by using global approaches have significantly enhanced our knowledge of the components involved in the transcriptional regulation of the cell cycle [1–3]. The Mcm1p-Fkh2p complex, in combination with the coactivator Ndd1p, plays an important role in the cell cycle-dependent expression of the *CLB2* gene cluster during the G2 and M phases ([4–7]; see [8–10] for reviews). Fkh2p is phosphorylated in a cell cycle-dependent manner, and peak phosphorylation occurs coincidentally with maximal expression of Mcm1p-Fkh2p-dependent gene expression [6]. However, the mechanism by which this complex is activated in a cell cycle-dependent manner is unknown. Here, we demonstrate that the forkhead-associated (FHA) domain of Fkh2p directs cell cycle-regulated transcription and that the activity of this domain is dependent on the coactivator Ndd1p. Ndd1p was found to be phosphorylated in a cell cycle-dependent manner by Cdc28p-Clb2p, and, importantly, this phosphorylation event promotes interactions between Ndd1p and the FHA domain of Fkh2p. Furthermore, mutation of the FHA domain blocks these phosphorylation-dependent interactions and abolishes transcriptional activity. Our data therefore link the transcriptional activity of the FHA domain with cell cycle-dependent phosphorylation of the coactivator Ndd1p and reveal a mechanism that permits precise temporal activation of the Mcm1p-Fkh2p complex.

*Correspondence: a.d.sharrocks@man.ac.uk (A.D.S.), b.a.morgan@ncl.ac.uk (B.A.M.)

[5]These authors contributed equally to this work.

Results and Discussion

The forkhead transcription factor Fkh2p is thought to act as an activator protein that drives the cell cycle-dependent expression of genes in the *CLB2* cluster ([4–7]; see [8–10] for reviews). To identify and map any transcriptional activation domain(s) (TAD) in Fkh2p, we created a series of fusion proteins with the Gal4p DNA binding domain, which spanned the length of Fkh2p (Figure 1A). These fusion proteins were then tested in a reporter gene assay by using a Gal4p binding site-dependent *lacZ* reporter gene. Initially, activities were measured in cells that had been synchronized in either G1 phase with α factor or M phase with nocodazole (Figure 1B). Of the three regions tested, strong transcriptional activity was only seen from the N-terminal part of Fkh2p (amino acids 1–254). Moreover, an increase in activity was apparent in nocodazole-treated cells compared to α factor-treated cells, and this suggests that the activity of this domain increases at later times during the cell cycle. To further investigate this increase in activity, the activity of Gal-Fkh2(1–254) was compared at different times in the cell cycle following release from α factor arrest (Figure 1C). Consistent with the analysis of nocodazole-treated cells, a gradual increase in the activity of Gal-Fkh2(1–254) was observed as cells progress through the cycle, and the increase of activity occurs at the time when enhanced activity of the Mcm1p-Fkh2p complex is predicted to occur [6]. Thus, these data demonstrate that amino acids 1–254 of Fkh2p contain a cell cycle-regulated TAD.

Cdc28p-Clb2p is important for the cell cycle-dependent expression of genes in the *CLB2* gene cluster [11]. Hence, it was possible that the activity of the Fkh2p TAD is regulated through cyclin-dependent kinase (Cdk)-mediated phosphorylation. However, although this region of Fkh2p lacks potential Cdk sites, it does contain a FHA domain that is known to act as a phosphothreonine binding motif in other proteins (reviewed in [12]). Thus, it is possible that the FHA domain provides a link to cell cycle-dependent phosphorylation events. The arginine residue at position 87, which is critical for phosphopeptide binding in other proteins (reviewed in [12]), was therefore mutated to alanine (R87A) to determine whether this was important for its transcriptional activation capacity. In contrast to the high transcriptional activity of the wild-type motif, the activity of Gal-Fkh2(1–254)(R87A) was severely compromised in vivo (Figure 1D). Furthermore, the transcriptional activation capacity of Gal-Fkh2(1–254)(R87A) was also compromised in in vitro transcription assays (see Figure S1 in the Supplemental Data available with this article online).

The putative coactivator protein, Ndd1p, has pre-

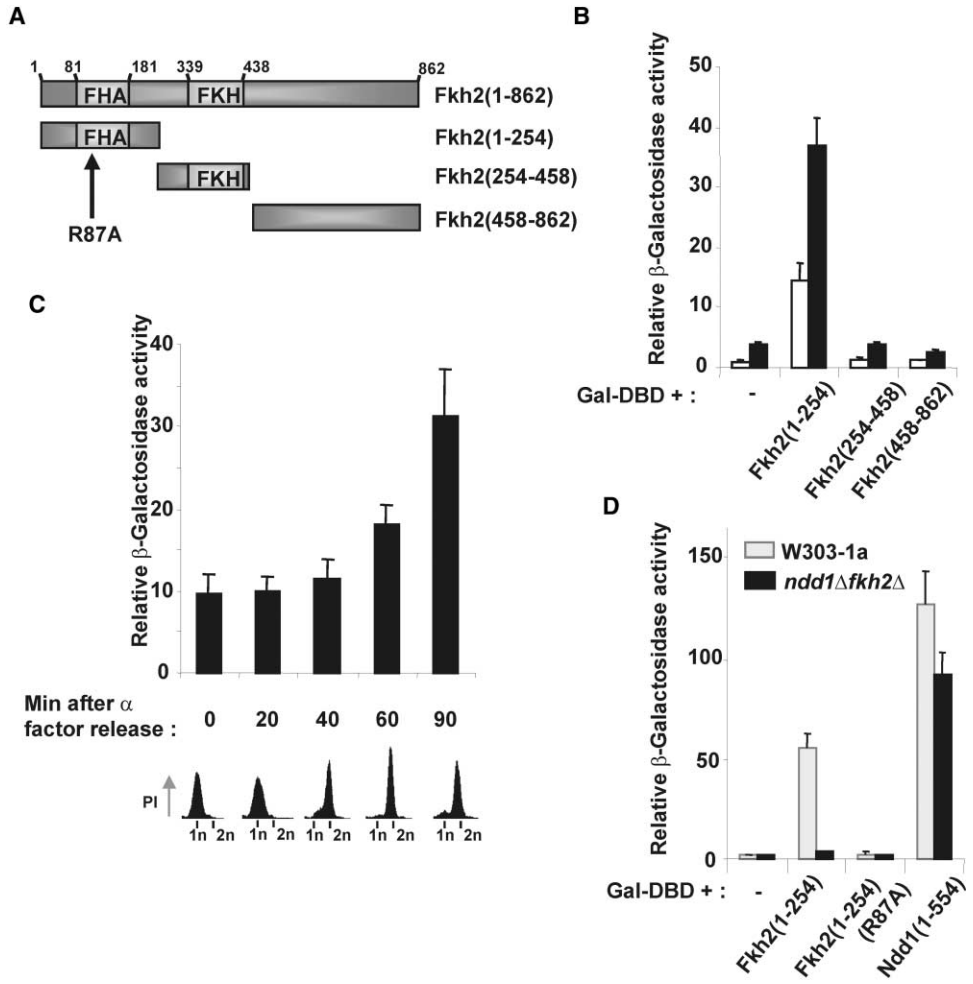

Figure 1. The FHA Domain of Fkh2p Is Part of a Cell Cycle-Regulated TAD

(A) A schematic representation of full-length Fkh2p and a series of deletion constructs. The locations of the Forkhead DNA binding (FKH) and FHA domains and the position of the R87A substitution are indicated.

(B and C) *MEL-Lac*Z reporter gene analysis with the indicated fusions of Fkh2p to the Gal4p DNA binding domain (Gal-DBD) in AH109 cells. Samples were assayed (B) after cell cycle arrest with nocodazole (black bars) or α factor (white bars) or (C) after the indicated times following release from α factor arrest. Cell cycle profiles were determined by flow cytometry analysis of propidium iodide (PI)-labeled cells. The locations of peaks corresponding to 1n and 2n DNA content are shown in (C).

(D) *GAL-Lac*Z reporter gene analysis with the indicated fusions of Fkh2p or Ndd1p to the Gal4p DNA binding domain (Gal-DBD). Samples were assayed from logarithmically growing wild-type (W303-1a) or *ndd1*Δ*fkh2*Δ strains.

viously been shown to be recruited to promoters regulated by the Mcm1p-Fkh2p complex [4, 13] and is essential for the activation of the *CLB2* gene cluster [14]. As the Mcm1p-Fkh2p complex remains bound to promoters of the *CLB2* gene cluster throughout the cell cycle [2], the recruitment of additional factors such as Ndd1p might be a key activity of the Fkh2p TAD. We therefore tested whether the activity of the Fkh2p TAD was dependent on the presence of Ndd1p (Figure 1D). A requirement for Ndd1p was demonstrated, as the activity of the Gal-Fkh2(1–254) was reduced to near basal levels in cells lacking Ndd1p. The activity of a Gal-Ndd1p fusion protein was also tested in wild-type and *ndd1*Δ*fkh2*Δ cells and elicited only slightly reduced levels of activation in the *ndd1*Δ*fkh2*Δ cells; this finding demonstrates that a reciprocal requirement for Fkh2p is not observed (Figure 1D).

The requirement for both Ndd1p and a functional FHA domain for the transcriptional activity of Fkh2(1–254) suggests that Ndd1p might represent a phosphorylation-dependent protein that in turn acts through binding to the Fkh2p FHA domain. Hence, we studied the mobility of Ndd1p by SDS-PAGE to investigate whether there was a retarded, slower mobility species that is indicative of cell cycle-dependent phosphorylation. Extracts prepared from cells expressing HA-tagged Ndd1p were analyzed by Western blotting (Figure 2A). Consistent with previous observations [14], the levels of Ndd1p were regulated in a cell cycle-dependent manner, and peak levels were detectable 60 min after release from α factor

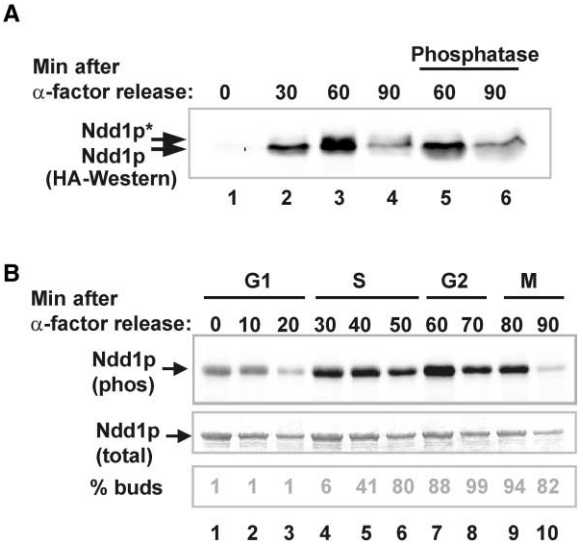

Figure 2. Ndd1p Is a Cell Cycle-Regulated Phosphoprotein

(A) Western blot analysis with HA antibody of extracts isolated from W303-1a cells, expressing HA-tagged Ndd1p, at the indicated times after release from an α factor block. The extracts in lanes 5 and 6 were treated with calf intestinal alkaline phosphatase. Bands corresponding to Ndd1p and a hyperphosphorylated slower-migrating form (asterisked) are indicated.

(B) Wild-type W303-1a cells were synchronized with α factor, and extracts were prepared from samples taken every 10 min following release from the block for precipitation of kinases by purified GST-Ndd1p. Kinase assays were then carried out for 20 min at 30°C and were analyzed by SDS-PAGE. Input levels of Ndd1p were determined by Coomassie staining (middle panel). A budding index is shown in the bottom panel.

arrest as cells enter the G2 phase (Figure 2A, lane 3). In addition, a lower-mobility species was detected after 60 min that was still detectable at a reduced level after 90 min (Figure 2A, lanes 3 and 4). Treatment of these extracts with phosphatase resolved the mobility of the bands back to their starting position at 30 min and thereby demonstrated that the reduced mobility was due to phosphorylation (Figure 2A, lanes 5 and 6).

To identify the cell cycle-regulated kinase responsible for Ndd1p phosphorylation, we first isolated Ndd1p-associated protein kinases at different times during the cell cycle. GST-Ndd1p fusion proteins were immobilized on agarose beads and were mixed with yeast cell extracts taken at various times following α factor arrest and release (Figure 2B). Following washing of the beads, kinase assays were then initiated by the addition of radioactively labeled ATP to reveal the presence of any Ndd1p-associated active protein kinases (Figure 2B). Low-level phosphorylation was detected in extracts from α factor-arrested cells (Figure 2B, lane 1). However, upon entry into S phase, phosphorylation levels increased and remained high in G2 (Figure 2B, lanes 4–9). Kinase activity then declined back to basal levels in M phase (Figure 2B, lane 10).

The timing of the cell cycle-dependent phosphorylation of Ndd1p suggests that it is targeted by a Cdc28p-Clbp complex. Indeed, analysis of the predicted sequence of Ndd1p revealed the presence of several potential Cdc28p phosphorylation sites conforming to the minimum Cdk recognition sequence Ser/ThrPro [15] (see below). Hence, we next examined whether any of the Clbp cyclins were important for Ndd1p phosphorylation. Firstly, we analyzed the activity of the Ndd1p-associated kinase from wild-type (W303-1a), *clb5*Δ, or *clb6*Δ cells that had been blocked in G1 by α factor or in M phase by nocodazole. Little kinase activity was associated with Ndd1p in G1 extracts from all three strains (Figure 3A, lanes 1–3), whereas high levels of kinase activity were observed in nocodazole-blocked cells (Figure 3A, lanes 4–6). As Ndd1p phosphorylation is not dependent on either Clb5p or Clb6p, we next focused on Clb1p–Clb4p, which act later in the cell cycle. In particular, we wished to study the potential role of Clb2p in Ndd1p phosphorylation, as this has been implicated in regulating its own expression by a positive feedback mechanism [11]. Hence, the Ndd1p precipitation experiment was repeated by using extracts isolated from *clb1*Δ*clb2*^{ts} *clb3*Δ*clb4*Δ mutant cells grown at the permissive and restrictive temperatures (Figure 3B). The immobilized Ndd1p only precipitated highly active protein kinases from extracts prepared from *clb1*Δ*clb2*^{ts}*clb3*Δ*clb4*Δ cells grown at the permissive temperature (Figure 3B). In contrast to the decrease in phosphorylation of Ndd1p, no change in the activity of the Fkh2p-associated kinase complex was observed in extracts taken from *clb1*Δ *clb2*^{ts}*clb3*Δ*clb4*Δ cells at the restrictive temperature (Figure 3B, lanes 4–6). Collectively, these data demonstrate that Clb2p has a major role in cell cycle-dependent phosphorylation of Ndd1p.

To demonstrate that Clb2p-containing complexes are directly responsible for Ndd1p phosphorylation rather than a kinase regulated by Clb2p-kinase complexes, we examined whether the kinase complex(es) precipitated by Ndd1p was thermolabile in an in vitro kinase assay. Extracts were isolated from wild-type (W303-1a) or *clb1*Δ*clb2*^{ts}*clb3*Δ*clb4*Δ cells grown at the permissive temperature (and hence contain active Clb2p) and were used for kinase reactions either at the usual temperature, 30°C, or at 37°C to inactivate Clb2p (Figure 3C). Efficient phosphorylation of Ndd1p was observed at both reaction temperatures by using extracts isolated from wild-type cells (Figure 3C, lanes 1 and 3). In contrast, phosphorylation of Ndd1p was greatly reduced in vitro when extracts isolated from the *clb1*Δ*clb2*^{ts}*clb3*Δ *clb4*Δ strain were used and incubated at 37°C (Figure 3C, lanes 2 and 4). Furthermore, Ndd1p and Clb2p can be coimmunoprecipitated from yeast extracts, demonstrating that they interact in vivo (see Figure S2 in the Supplemental Data). To further demonstrate the importance of Clb2p in enhancing Ndd1p phosphorylation in vivo, *CLB2* was overexpressed in wild-type cells transformed with an HA-tagged Ndd1p expression vector, and the mobility of Ndd1p was analyzed on a Western blot. In logarithmically growing cells, overexpression of *CLB2* led to enhancement of a lower-mobility band, thereby demonstrating enhanced Ndd1p phosphorylation (Figure 3D, lane 2). To investigate whether Cdc28p-Clb2p can directly phosphorylate Ndd1p, kinase assays were carried out in vitro with specific cyclin-Cdc28p

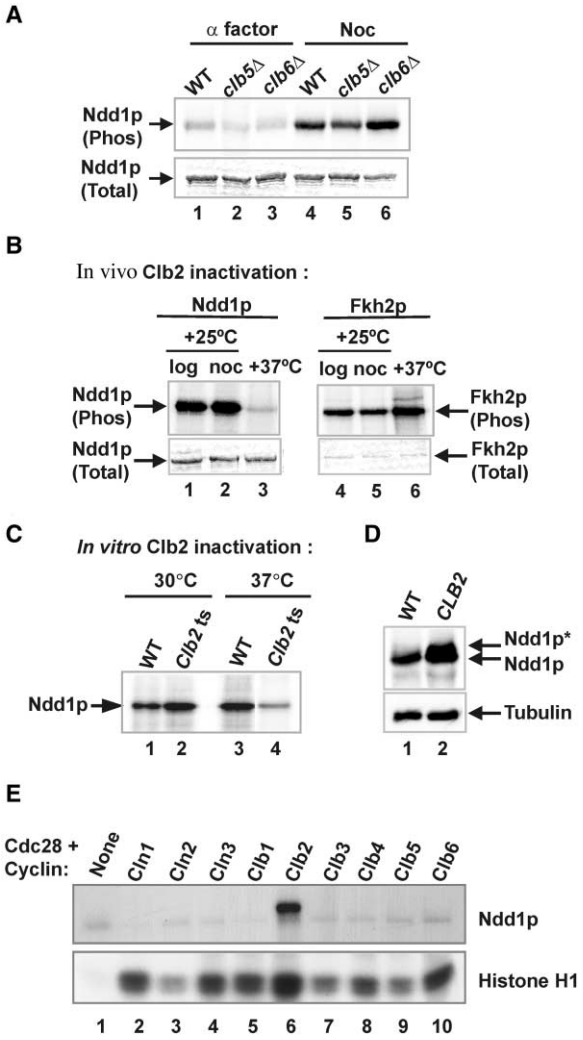

Figure 3. Ndd1p Is Phosphorylated by the Cdc28p-Clb2p Kinase

(A) Ndd1p immobilized on glutathione-agarose beads was incubated with extracts isolated from wild-type ([W303-1a], WT, lanes 1 and 4), *clb5*Δ (lanes 2 and 5), or *clb6*Δ (lanes 3 and 6) cells synchronized with α factor or nocodazole (noc). Kinase reactions were then carried out with the precipitated kinases, and the assays were analyzed by SDS-PAGE.

(B) SDS-PAGE analysis of in vitro kinase assays of Ndd1p and Fkh2p by coprecipitated kinases by using extracts isolated from cells of the *clb1*Δ*clb2*^{ts}*clb3*Δ*clb4*Δ strain grown at 25°C that were untreated (log; lanes 1 and 4), treated with nocodazole (noc; lanes 2 and 5), or shifted to the restrictive temperature (37°C; lanes 3 and 6). The total levels of Fkh2p and Ndd1p in these assays were determined by Coomassie staining (lower panels of [A] and [B]).

(C) In vitro kinase assays were performed with Ndd1p used to coprecipitate kinases from extracts isolated from wild-type ([W303-1a], WT) or *clb1*Δ*clb2*^{ts}*clb3*Δ*clb4*Δ (Clb2 ts) cells grown at 25°C. Kinase assays were carried out at 30°C or 37°C and were analyzed by SDS-PAGE.

(D) Western blot analysis with HA antibody of extracts isolated from W303-1a cells expressing HA-tagged Ndd1p (WT; lane 1). In lane 2, cells were additionally transformed with a vector (pMW20-Clb2-His_6Flag) encoding Clb2p (*CLB2*; lane 2). Extracts were prepared from logarithmically growing cells. Bands corresponding to Ndd1p and a hyperphosphorylated slower-migrating form (asterisked) are indicated. The lower panel shows a control Western blot of tubulin levels.

(E) Cdc28p isolated from yeast was assayed to phosphorylate bacterially expressed GST-Ndd1p (top panel) and histone H1 (bottom panel) in the absence of any supplementary cyclin (lane 1) or in the presence of the indicated cyclins purified from bacteria (lanes 2–10).

kinase complexes. GST-Cdc28p was affinity purified from yeast under conditions that minimize copurification of endogenous cyclins, and each of nine different purified cyclins was added. Little kinase activity toward either histone H1 or Ndd1p was detected in the absence of supplementary cyclins (Figure 3E, lane 1). However, although all nine cyclin-Cdc28p complexes were active toward histone H1, only Cdc28p in combination with Clb2p stimulated phosphorylation of Ndd1p (Figure 3E, lane 6, top panel). Consistent with this observation, immunoprecipitated, Flag-tagged Clb2p (see Figure S3 in the Supplemental Data) and HA-tagged Cdc28p complexes (data not shown) could also phosphorylate Ndd1p. Thus, although we cannot formally rule out a potential additional role for Clb1p, Clb3p, and Clb4p in Ndd1p phosphorylation in vivo, our data demonstrate that the Cdc28p-Clb2p complex plays a major, direct role in Ndd1p phosphorylation during the cell cycle.

In order to map where Cdc28p-Clb2p phosphorylates Ndd1p, GST fusion proteins were created containing either the N (amino acids 1–250) or C (amino acids 247–554) terminus of Ndd1p and were tested as potential substrates for the Cdc28p-Clb2p kinase. Phosphorylation of both Ndd1(1–250) and Ndd1(247–554) was observed (Figure S3). However, phosphorylation of Ndd1(247–554) was stronger, indicating that the majority of sites are located in the C-terminal part of the protein.

Next, we tested the ability of Clb2p-mediated Ndd1p phosphorylation to promote interactions with the N-terminal part of Fkh2p encompassing amino acids 1–458. To probe the potential role of the FHA domain and its phosphopeptide binding capability on Ndd1p interactions, we also analyzed the R87A version of Fkh2(1–458). In the absence of phosphorylation, full-length Ndd1p bound weakly to wild-type and mutant versions of Fkh2(1–458) (Figure 4B, top panel, lanes 1 and 2). However, upon Clb2p-mediated phosphorylation of Ndd1p, enhanced binding to the wild-type Fkh2(1–458) was observed (Figure 4B, bottom panel, lanes 1 and 2). To localize the region(s) of Ndd1p responsible for this phosphorylation-enhanced binding, we compared the ability of the N- and C-terminal regions of Ndd1p to interact with the Fkh2(1–458) constructs. The C-terminal part of Ndd1p (amino acids 247–554) behaved in a similar manner to full-length Ndd1p, with low basal levels of binding that were only significantly stimulated by Clb2p-mediated Ndd1p phosphorylation in conjunction with wild-type Fkh2p, and not the R87A derivative (Figure 4B, lanes 5 and 6). In contrast to GST-Ndd1(1-554) and GST-Ndd1(247–554), little detectable binding of the N-terminal region of Ndd1p in GST-Ndd1(1–250) to Fkh2(1–458) was observed in the absence of phosphorylation (Figure 4B, top panel, lanes 3 and 4). Upon phosphorylation, strong binding of GST-Ndd1(1–250) was observed to wild-type Fkh2(1–458) (Figure 4B, bottom panel, lane 3). However, little binding of the phosphorylated Ndd1(1–250) was detected with the R87A form of Fkh2(1–458) (Figure 4B, bottom panel, lane 4). Thus,

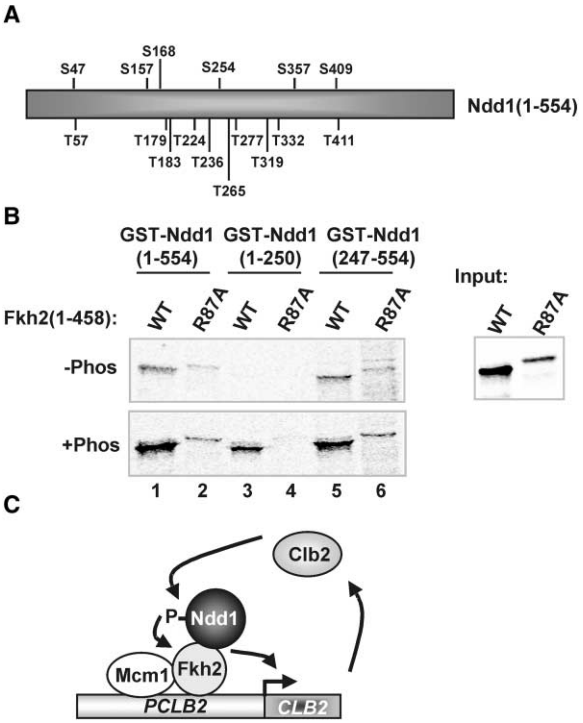

Figure 4. Phosphorylation-Dependent Regulation of Intermolecular Interactions between Ndd1p and the FHA Domain of Fkh2p

(A) A schematic representation of full-length Ndd1p showing the locations of potential Cdk (Ser/ThrPro) phosphorylation sites.

(B) GST pull-downs of the indicated GST-Ndd1p constructs and in vitro-translated Fkh2(1–458) constructs. GST fusion proteins were phosphorylated by immunoprecipitated Clb2p-kinase complexes (+Phos) or were left unphosphorylated (−Phos) before carrying out the pull-down assay. The top and bottom panels were exposed for equivalent amounts of time. The inputs of the Fkh2(1–458) constructs (20%) is shown. Note that Fkh2(1–458)(R87A) contains an additional myc-epitope tag.

(C) The cartoon depicts the Mcm1p-Fkh2p-Ndd1p complex that forms on the promoters of genes in the *CLB2* cluster. Clb2p acts in a positive feedback loop to potentiate the activation of expression of these genes by phosphorylating and activating the Ndd1p subunit of this complex.

optimum binding of the N- and C-terminal regions of Ndd1p to the N-terminal region of Fkh2p requires both Clb2p-mediated phosphorylation of Ndd1p and the ability of the FHA domain of Fkh2p to bind to phosphopeptides.

In summary, these findings strongly suggest that the cell cycle-dependent activity of the TAD of Fkh2p is linked by Clb2p-regulated interactions with Ndd1p and provide evidence for an elegant molecular switch that controls the activity of Fkh2p during the cell cycle. Clb2p has been shown to be required for the cell cycle-dependent expression of several genes in the *CLB2* gene cluster via a positive feedback loop [11]. Similarly, we have shown that the activity of the *SWI5* promoter is also dependent on Clb2p (see Figure S4 in the Supplemental Data). The data presented here provide evidence for the molecular mechanism underlying this positive feedback loop (see Figure 4C). Moreover, our data are consistent with a recent study that also identified phosphorylation-dependent binding of Ndd1p to the FHA domain of Fkh2p [16]. Importantly, however, we extend these findings by demonstrating that Ndd1p is a direct target of the Cdc28p-Clb2p kinase and that Fkh2p and Ndd1p interact directly through the FHA domain of Fkh2p.

Previously, Fkh2p was also shown to be phosphorylated in a cell cycle-dependent manner [6]. Although there are no potential Cdk sites in the Fkh2p TAD, we have mapped sites in the C terminus of Fkh2p that are phosphorylated in a Clbp-dependent manner and that are also important for interactions with Ndd1p (unpublished data). Thus, taken together, these data suggest that the integration of different cell cycle-dependent phosphorylation events on two different components of the Mcm1p-Fkh2p-Ndd1p complex permits its precise temporal activation and the ensuing expression of the *CLB2* gene cluster. Such combinatorial events likely converge on other transcription factor complexes in yeast and higher eukaryotes to permit precise control of gene expression during the cell cycle.

Supplemental Data

Supplemental Data including Figures S1–S4 and a more detailed description of the Experimental Procedures used in this study are available at http://www.current-biology.com/cgi/content/full/13/19/1740/DC1/.

Acknowledgments

We would like to thank Anne Clancy and June Saunders for excellent technical assistance, Peter O'Callaghan for help with the FACS analysis, David Lydall and members of our laboratories for comments on the manuscript and helpful discussions, and Lee Johnston, David Lydall, and Uttam Surana for reagents. This work was supported by Cancer Research UK, the Wellcome Trust, the Lister Institute of Preventive Medicine, and the Medical Research Council.

Received: June 30, 2003
Revised: August 4, 2003
Accepted: August 25, 2003
Published online: September 4, 2003

References

1. Cho, R.J., Campbell, M.J., Winzeler, E.A., Steinmetz, L., Conway, A., Wodicka, L., Wolfsberg, T.G., Gabrielian, A.E., Landsman, D., Lockhart, D.J., et al. (1998). A genome-wide transcriptional analysis of the mitotic cell cycle. Mol. Cell *2*, 65–73.
2. Simon, I., Barnett, J., Hannett, N., Harbison, C.T., Rinaldi, N.J., Volkert, T.L., Wyrick, J.J., Zeitlinger, J., Gifford, D.K., Jaakkola, T.S., et al. (2001). Serial regulation of transcriptional regulators in the yeast cell cycle. Cell *106*, 697–708.
3. Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M.B., Brown, P.O., Botstein, D., and Futcher, B. (1998). Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. Mol. Biol. Cell *9*, 3273–3297.
4. Koranda, M., Schleiffer, A., Endler, L., and Ammerer, G. (2000). Forkhead-like transcription factors recruit Ndd1 to the chromatin of G2/M-specific promoters. Nature *406*, 94–98.
5. Kumar, R., Reynolds, D.M., Shevchenko, A., Shevchenko, A., Goldstone, S.D., and Dalton, S. (2000). Forkhead transcription factors, Fkh1p and Fkh2p, collaborate with Mcm1p to control transcription required for M-phase. Curr. Biol. *10*, 896–906.
6. Pic, A., Lim, F.-L., Ross, S.J., Veal, E.A., Johnson, A.L., Sultan, M.R., West, A.G., Johnston, L.H., Sharrocks, A.D., and Morgan, B.A. (2000). The forkhead protein Fkh2 is a component of the yeast cell cycle transcription factor SFF. EMBO J. *19*, 3750–3761.
7. Zhu, G., Spellman, P.T., Volpe, T., Brown, P.O., Botstein, D.,

Davis, T.N., and Futcher, B. (2000). Two yeast forkhead genes regulate the cell cycle and pseudohyphal growth. Nature *406*, 90–94.

8. Breeden, L.L. (2000). Cyclin transcription: timing is everything. Curr. Biol. *10*, R586–R588.
9. Futcher, B. (2000). Microarrays and cell cycle transcription in yeast. Curr. Opin. Cell Biol. *12*, 710–715.
10. Jorgensen, P., and Tyers, M. (2000). The fork'ed path to mitosis. Genome Biol. *1*, 1022.1–1022.4.
11. Amon, A., Tyers, M., Futcher, B., and Nasmyth, K. (1993). Mechanisms that help the yeast cell cycle clock tick: G2 cyclins transcriptionally activate G2 cyclins and repress G1 cyclins. Cell *74*, 993–1007.
12. Durocher, D., and Jackson, S.P. (2002). The FHA domain. FEBS Lett. *513*, 58–66.
13. Lydall, D., Ammerer, G., and Nasmyth, K. (1991). A new role for MCM1 in yeast: cell cycle regulation of SWI5 transcription. Genes Dev. *5*, 2405–2419.
14. Loy, C.J., Lydall, D., and Surana, U. (1999). NDD1, a high-dosage suppressor of cdc28–1N, is essential for expression of a subset of late-S-phase-specific genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *19*, 3312–3327.
15. Songyang, Z., Blechner, S., Hoagland, N., Hoekstra, M.F., Piwnica-Worms, H., and Cantley, L.C. (1994). Use of an oriented peptide library to determine the optimal substrates of protein kinases. Curr. Biol. *4*, 973–982.
16. Reynolds, D., Shi, B.J., McLean, C., Katsis, F., Kemp, B., and Dalton, S. (2003). Recruitment of Thr 319-phosphorylated Ndd1p to the FHA domain of Fkh2p requires Clb kinase activity: a mechanism for *CLB* cluster gene activation. Genes Dev. *17*, 1789–1802.